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Rethinking cycad metabolite research

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Cycads are among the most ancient of extant Spermatophytes, and are known for their numerous pharmacologically active compounds. One compound in particular, β-methylamino-L-alanine (BMAA), has been implicated as the cause of amyotrophic lateral sclerosis/Parkinson dementia complex (ALS/PDC) on Guam. Previous studies allege that BMAA is produced exclusively by cyanobacteria, and is transferred to cycads through the symbiotic relationship between these cyanobacteria and the roots of cycads. We recently published data showing that *Cycas micronesica* seedlings grown without endophytic cyanobacteria do in fact increase in BMAA, invalidating the foundation of the BMAA hypothesis. We use this example to suggest that the frenzy centered on BMAA and other single putative toxins has hindered progress. The long list of cycad-specific compounds may have important roles in signaling or communication, but these possibilities have been neglected during decades of attempts to force single metabolites into a supposed anti-herbivory function. We propose that an unbiased, comprehensive approach may be a more appropriate means of proceeding with cycad biochemistry research.

Key words: BMAA, chromatography, Cycadaceae, *Cycas micronesica*, mass spectrometry, metabolomics

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Introduction

Cycads have been known for their toxic properties for centuries, and reports dating back to the 1770s described severe illnesses and gastrointestinal disturbances that can result from ingestion. Nevertheless, cycads were a documented food source among certain native populations including Australia, Fiji and Guam.[1,2]

In the 1950s, a unique combination of neurodegenerative diseases was discovered among the native Guamanian population. This condition was referred to as amyotrophic lateral sclerosis/Parkinson dementia complex (ALS/PDC) and the intense research that followed showed the disease rates to be consistent with some unknown combination of genetic and environmental factors.[3-6] Ingestion of foods prepared from cycads was singled out early on as a possible cause of ALS/PDC since cycads produce a variety of toxic substances. This causal link was confirmed decades later.[7]

Among the studied toxins are cycasin,[8] steryl glucosides[9] and β-methylamino-L-alanine (BMAA), a non-protein amino acid.[10] Moderate doses of BMAA produce no neurological or behavioral effects on animals, and high doses cause acute toxicity; neither of these scenarios mimic the gradual onset of ALS/PDC in humans.[11] These inconsistent results and the lack of a BMAA animal model for ALS/PDC have been ignored to sustain BMAA as the environmental trigger for ALS/PDC and other neurological conditions.[12-14] BMAA has been quantified in several samples types,[15-18] yet none of these studies has attempted to fully examine the origin of BMAA, its role in cycad biology, or the precise role of cyanobacteria in the symbiotic relationship with cycads. Furthermore, attempts to reproduce the detection of BMAA in human tissues have not been successful, despite the use of validated methods.[19,20]

Cyanobacteria cultured from cycad roots have been found to contain BMAA, but we do not regard this as evidence that

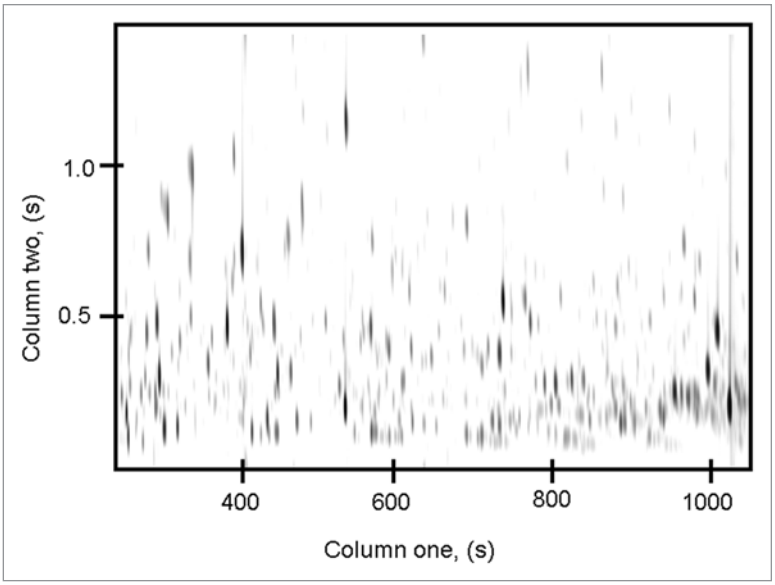

Figure 1. A representative chromatographic separation of a *Cycas micronesica* leaf sample displaying a large number of peaks. Each of these peaks corresponds to a compound which illustrates the chemical complexity of the cycad metabolome.

BMAA was produced by the endosymbiont. An equally plausible explanation was that BMAA was synthesized by cycad tissue then transferred to the endosymbiont. In that light, we recently published the first study that directly determines if endophytic cyanobacteria are required for biosynthesis of BMAA in cycads,[21] and the results clearly verified an increase in BMAA occurred without cyanobacteria symbiosis.

We offer several lines of reasoning for re-thinking the approach to studying cycad toxins. First, a plethora of secondary compounds are present in cycads,[22] and little is known about the biological significance of these molecules. The function of each plant metabolite is never expressed in isolation, but instead acts in tandem with other molecules present within the cornucopia of the "metabolome." We view the "tunnel vision" approach of ignoring the many co-occurring metabolites as an example of Ockham's broom.[23] Second, the primary role of putative or proven toxins may be physiological or signaling,[24] with mammalian toxicity being an incidental trait. Indeed, our results indicated BMAA was heavily concentrated in cycad roots,[21] which does not support the notion that the primary role of BMAA is an anti-herbivore compound as proclaimed by advocates of the BMAA hypothesis. Third, more recent work has indicated that uncharacterized toxins, excluding BMAA, may play a role in the development of cycad-induced disease.[25,26]

Comprehensive Analysis

"Metabolomics" is the comprehensive analysis of small molecules present in biological specimens that reflects a particular condition or phenotype. This type of analysis is mainly concerned with the identification of small organic compounds such as amino acids, sugars and fatty acids, and has already been used to characterize a variety of biological specimens.[27-30]

While several techniques can be used to assess the metabolome of a sample, a single analytical technique that enables the analysis of all metabolites present would be ideal. For example, multi-dimensional chromatography methods, in particular comprehensive two-dimensional gas chromatography coupled with time-of-flight-mass-spectrometry analysis (GC x GC-TOFMS), have been developed to offer a more complete picture of an organism's metabolome. A two dimensional chromatographic separation is attained by employing complementary stationary phases,[31-33] and compounds are identified using two unique retention times as well as mass spectral data. The advantages of this recent technology are increased sensitivity and greater utilization of separation space, which helps alleviate the problem of co-elution in complex samples. A representative separation using this technique for a *Cycas micronesica* leaf sample reveals the plethora of compounds produced by this species (**Fig. 1**).

Conclusions

Chasing single metabolites such as BMAA has been the traditional and expensive approach of cycad toxicity research for decades; copious funds and many years have been wasted as a result. This approach has led to several dead-ends and engendered the fabrication of elaborate biomagnification proposals involving several trophic levels. Our simple study,[21] which could have been conducted early on, invalidated the foundation of the BMAA hypothesis after years of research devoted to prove its legitimacy.

These attempts to force single metabolites into an anti-herbivory function have hindered progress in uncovering more realistic functions of the collective cycad metabolome, such as signaling.[24] Indeed, the high concentration of BMAA in coralloid roots[18] is consistent with a communication role where it may initiate or sustain the symbiotic relations of cycad roots and endosymbionts. Unfortunately, this type of thinking has not yet been applied to cycad chemistry research. Considering that cycads are the most threatened group of plant species on Earth, and some cycad chemicals are not found in any other plant group,[34] time may be running out. Perhaps it is time to retire the use of Okham's broom that is used to ignore the metabolome while chasing single metabolites. We opine that the study of cycad chemistry should at least temporarily change course and adopt a comprehensive metabolomics approach.

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